Ly-6 in Kidney Is Widely Expressed on Tubular Epithelium and Vascular Endothelium and Is Up-Regulated by Interferon Gamma[1,2]

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ABSTRACT

Ly-6 is a multigene family of murine polymorphic cell membrane proteins that are glycosydiphosphatidylinositol anchored, widely expressed on lymphoid tissue, and homologous to the recently described human CD59. An unexpected feature of Ly-6 is its high level of expression in the kidney. This renal expression and its interferon (IFN)-γ inducibility in murine strains expressing different Ly-6 haplotypes were studied with monoclonal antibodies and cDNA probes that recognize Ly-6A/E and Ly-6C. Ly-6 expression was much more extensive in the kidney than in other parenchymal organs. Ly-6A.1/E.2 was extensively expressed on vascular endothelium and on tubular epithelium, particularly in the distal nephron. Pattern of expression differed between strains expressing A and E alleles. Ly-6C was not detected by monoclonal antibodies but was detected by oligonucleotide-specific probes. Treatment with recombinant IFN-γ or IFN-inducing agents increased Ly-6 expression markedly, particularly on the luminal aspect of the proximal tubular epithelium, where Ly-6A/E became prominent. This luminal expression is typical for glycosydiphosphatidylinositol-anchored proteins but contrasts with that of other molecules, such as major histocompatibility classes I and II, which are generally expressed on the basolateral surface of the tubular epithelium. Up-regulation occurred within 6 h of IFN-γ treatment and returned to normal by 48 h. Similar up-regulation of Ly-6 was seen in murine lupus nephritis and in mercuric chloride nephropathy. The characteristics of renal Ly-6, such as its IFN-γ responsiveness, endothelial and tubular expression, polymorphism, strong antigenicity, and possible allelic regulation, make it a candidate to be a target molecule in alloresponses. The renal expression of Ly-6 is similar to that of CD59 in the human kidney, supporting the suggestion that these proteins are closely related.

Key Words: *CD59, lymphocyte activation, glycophosphatidylinositol, lupus nephritis, mercuric chloride nephropathy*

Ly-6 is a multigene family of murine polymorphic cell membrane proteins (1,2). It was first defined by the use of alloantibodies in 1977 (3), but in retrospect, many alloantisera against these highly antigenic proteins had been previously described (4–7). Two principal haplotypes have been identified and named Ly-6.1 and Ly-6.2 (8). Ly-6 molecules are 10- to 18-kd glycoproteins (9–11) and belong to the structurally and functionally diverse "family" of molecules linked to the cell membrane by a glycophosphatidylinositol (GPI) anchor (12–14). In recent years, DNA sequences of four of the Ly-6 genes have been reported and show over 80% homology with each other (15–19). Ly-6 proteins are expressed on B and T lymphocytes, where they are up-regulated by interferon (IFN)-γ (20,21). Monoclonal antibodies (mAb) against members of the Ly-6 family activate T cells when cross-linked (22,23), provided that there is T cell receptor expression (24). Conversely, Ly-6 expression appears to be required for T cell receptor–mediated activation (25,26). This suggests a role for Ly-6 in lymphocyte activation, but whether this function is physiologic is unclear.

Extralymphoid and, in particular, renal expression of Ly-6 was first noted over a decade ago with tissue absorption of antisera (3,27). It has since been confirmed by other groups but has not been well characterized (16,28). This renal expression raises the possibility of functions for Ly-6 on non-bone marrow–derived cells. Substantial homologies between the DNA sequences for Ly-6 and those for human CD59 (29,30) and urokinase-type plasminogen activator receptor (uPAR) (31) have recently been identi-

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fied. CD59, which is a homologous restriction factor for the membrane attack complex of complement, is of particular interest in that it has been shown to have extensive expression in the kidney, suggesting that it might be the human analog of Ly-6 (32–34).

In this study, we have used cDNA probes and mAb to examine the distribution and characteristics of Ly-6 in the kidney, particularly its IFN-γ responsiveness and its alteration in murine models of nephritis. We found that there is extensive renal expression of Ly-6 both on the vascular endothelium and on the tubular epithelium, particularly in the distal nephron, and that the pattern of tubular expression varies between strains with the two different Ly-6 haplotypes. Expression is further increased by IFN-γ, particularly on the proximal tubular epithelium where it becomes apparent in a luminal pattern. Renal Ly-6 is shown to be similarly up-regulated in murine lupus nephritis and in mercuric chloride nephropathy. The characteristics of renal Ly-6 are compared with those of CD59 in the human kidney.

METHODS

Mice

BALB/c (H-2^d), CAF1 [(BALB/c × A)F1] (H-2$^{d/a}$), and CBA/J (H-2^k) mice, which express the Ly-6.1 haplotype, and DBA/2J (H-2^d) mice, which express the Ly-6.2 haplotype, were housed at our animal colony. A.D2-Ly-6b (H-2^k) mice, which are congenic with strain A/J at the Ly-6 locus and express the Ly-6.2 haplotype, were produced, bred, and housed at our animal colony. MRL lpr/lpr mice, which carry the autosomal recessive lymphoproliferative (lpr) mutation (35), and MRL +/+ mice, which do not, were purchased from the Jackson Laboratory (Bar Harbor, ME) and housed at our animal colony. All mice were between 6 and 14 wk of age during the experiments.

Antibodies

YE3, a rat immunoglobulin (Ig)G mAb to an Ly-6 A/E determinant, was provided by Dr. Fumio Takei (B.C. Cancer Research Centre, Vancouver, Canada) (36). The specificity of this mAb has been clearly established (11). Ly-6A.2 and E.1 are considered to be alleles at the same locus, with the former being expressed by Ly-6.2 and the latter by Ly-6.1 murine strains, respectively (1,2). 34-11-3 and 34-2-11, murine IgG mAbs to Ly-6A.2 and Ly-6C.2, respectively, were provided by Dr. David Sachs *et al.* (37). The specificity of these two mAb has also been clearly established (37). M1, a rat-antimurine class I IgG mAb, and 343-4-205 and 11-4.1, murine-antimurine IgG mAb against H-2D and H-2K class I, respectively, were obtained from the American Type Culture Collection (ATCC; Bethesda, MD). The rat and mouse anti-class I mAb all react with classical mouse class I molecules and have no known reactivity with the class I–like molecules (Qa, T1a).

INF-γ Induction

INF-γ induction was done by the direct administration of recombinant mouse IFN-γ (100,000 U ip; supplied by Dr. Peter Van Der Meide, Primate Centre TNO, Rijswijk, Netherlands) (38). Mice were euthanized at 6, 24, and 48 h, and kidneys were harvested for RNA extraction and mAb staining. In addition, the effect of agents known to induce IFN production *in vivo* in the context of different immune responses was investigated (39). The latter were: (*1*) S. Minnesota lipopolysaccharide (Sigma Chemical Co., St. Louis, MO), which induces both IFN-α/β and INF-γ in a T cell–independent manner (40); (*2*) polyinosinic-polycytidylic acid (Sigma Chemical Co.), which induces IFN-α/β in a manner resembling viral infection (41); (*3*) oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one) (Sigma Chemical Co.), which induces IFN-γ in a delayed-type hypersensitivity-like response (42); (*4*) DBA/2 mastocytoma P815 cells, which were obtained from ATCC and maintained by weekly passage in female DBA/2 mice in ascitic form and which also induce IFN-γ in an allogeneic rejection response (41). Control mice were treated with topical acetone and ip phosphate-buffered saline (PBS). These agents were administered according to the protocols outlined in Table 1.

Radiation

Female CBA/J mice were subjected to whole-body gamma radiation doses of 500, 750, 1,000, 1,500, and 2,000 rads on Day 1 and were euthanized on Day five. Kidneys were harvested for RNA extraction.

TABLE 1. Protocols for agent administration

Oxazolone
100 μL of 25% oxazolone in acetone topically to shaven back on Day 1 and 25 μL of 5% oxazolone in acetone topically to shaven back on Day 4
Euthanized Day 6
Lipopolysaccharide
25 μg ip Days 1 and 4
Euthanized Day 6
Polyinosinic-Polycytidylic Acid
100 μg ip Day 1
Euthanized Day 4
P815
20 million cells ip Day 1
Euthanized Day 4
Control
Topical acetone to shaven back Days 1 and 4
PBS, 0.1 mL ip Days 1 and 4
Euthanized Day 6

Murine Models of Nephritis

Two murine models of nephritis were used—the spontaneous lupus-like illness of MRL lpr/lpr mice (35) and the induction of autoimmune nephritis by mercuric chloride (43). In the former, MRL lpr/lpr and control MRL +/+ mice were euthanized at 6 and 17 wk and kidneys were harvested for frozen sectioning and immunoperoxidase staining and for RNA extraction. In the latter, CBA/J mice were administered mercuric chloride (Sigma Chemical Co.) at doses of 1.6, 2.0, and 3.2 mg/kg body wt three times weekly as previously described (43). Mercuric chloride was injected ip in a 0.4% solution in 0.9% sterile saline. Control mice were injected ip with 0.9% sterile saline. Both treated and control mice were euthanized at 1 wk, at which time the induction of IFN-regulated major histocompatability complex (MHC) molecules has been shown to be maximal (43). Kidneys were then harvested for immunoperoxidase staining and RNA extraction.

Indirect Immunoperoxidase Staining

Because the epitopes recognized by all of these antibodies are lost after formaldehyde fixation, indirect immunoperoxidase (IIP) staining was done by techniques as previously described (39). Briefly, sections of fresh frozen kidney were fixed in acetone, incubated with normal goat serum for 20 min, washed three times in PBS, and incubated for 60 min with the first or test antibody. Sections were then incubated with the second antibody, either peroxidase-labeled goat anti-rat IgG and normal mouse serum for 60 min, in the case of rat mAb or peroxidase-labeled goat anti-mouse IgG for 60 min, in the case of mouse mAb. The color was developed by reacting with 3'3 diaminobenzidine tetrachloride and hydrogen peroxide for 10 min before counterstaining with hematoxylin.

To check for nonspecific reactivity of the second antibody, kidney sections from all mice were stained by the same protocol, except that PBS was used instead of the first antibody. This provided a negative control. To exclude nonspecific reactivity of the first antibodies of the same isotype, staining with class I mAb of the same isotype was used as a second negative control for the kidney sections from normal mice. Kidney sections from IFN-treated mice were stained with class I mAb as a positive control. In all experiments, tissues from three or more mice in each experimental group were examined.

Analysis of RNA Specific for Ly-6

Total RNA was isolated from kidney and from other tissues by the guanidinium-cesium chloride method (44). In all experiments, pooled RNA from three or more mice was prepared. RNA concentrations were then determined by absorbance at 260 nm. Northern blots were prepared with 15 to 30 μg of total RNA, electrophoresed through a 1.5% agarose-2.2 M formaldehyde gel, and transferred to nitrocellulose filters. The blots were then examined with 0.7- and 0.8-kilobase cDNA probes for Ly-6A.2 and Ly-6C.2, respectively (supplied in pcEXV expression vectors by Dr. R. Palfree, Royal Victoria Hospital, Montreal, Quebec, Canada). These probes cross-hybridize and therefore cannot be used to distinguish Ly-6A/E and Ly-6C. A cDNA probe for either actin or glyceraldehyde phosphate dehydrogenase was used to control the amounts of RNA loaded when RNA from kidneys was being compared. However, when RNA from the kidney was being compared with RNA from other tissues or when radiation had been administered, these controls were no longer appropriate and ethidium bromide staining of the gel was used instead to control for RNA loading. All probes were labeled with ^{32}P. To determine relative increases in Ly-6–specific RNA, autoradiographs of the blots were analyzed by video densitometry with integration of the area under the curve, corrected for the values obtained with the control probe. To distinguish between Ly-6A and Ly-6C, specific oligonucleotide probes (from Dr. R. Palfree) were used. The Ly-6A probe comprised 44 bases (5'-CTAATATTGAAAGTATGGGGAGATCCTGGGT-ACTAAGGTCAACGT-3') concatemerized to give a 240-base-pair sequence. The Ly-6C probe comprised 30 bases (5'-GGTGTGCCAATCAAGGATCCTAACA T-CAGG-3'). These probes correspond to the sequences where Ly-6A and Ly-6C differ most.

RESULTS

Distribution of Ly-6 in Normal Kidneys

On IIP staining of kidneys from normal A.D2-Ly-6b mice, Ly-6A.2 expression was detected with both mAb (YE3 and 34-11-3) on the endothelium of arterioles and venules but not on that of glomeruli or capillaries (Figure 1). There was prominent expression on the epithelium of distal tubules and collecting ducts in the renal medulla and, to a lesser degree, in the renal cortex (Figures 1–3). Expression was most prominent in the outer zone of the medulla. The staining of distal tubular epithelial cells took on a diffuse cytoplasmic pattern. Proximal tubules were mainly negative but sometimes stained faintly in a brush border pattern. Interstitial cells were not stained by the Ly-6 mAb. A similar pattern was observed in DBA/2J mice, which also express Ly-6.2 haplotype.

In normal CAF1 and BALB/c mice, similar expression of Ly-6E.1 on the vascular endothelium was seen with the YE3 mAb. However, tubular staining differed from that of Ly-6A.2 in being almost entirely

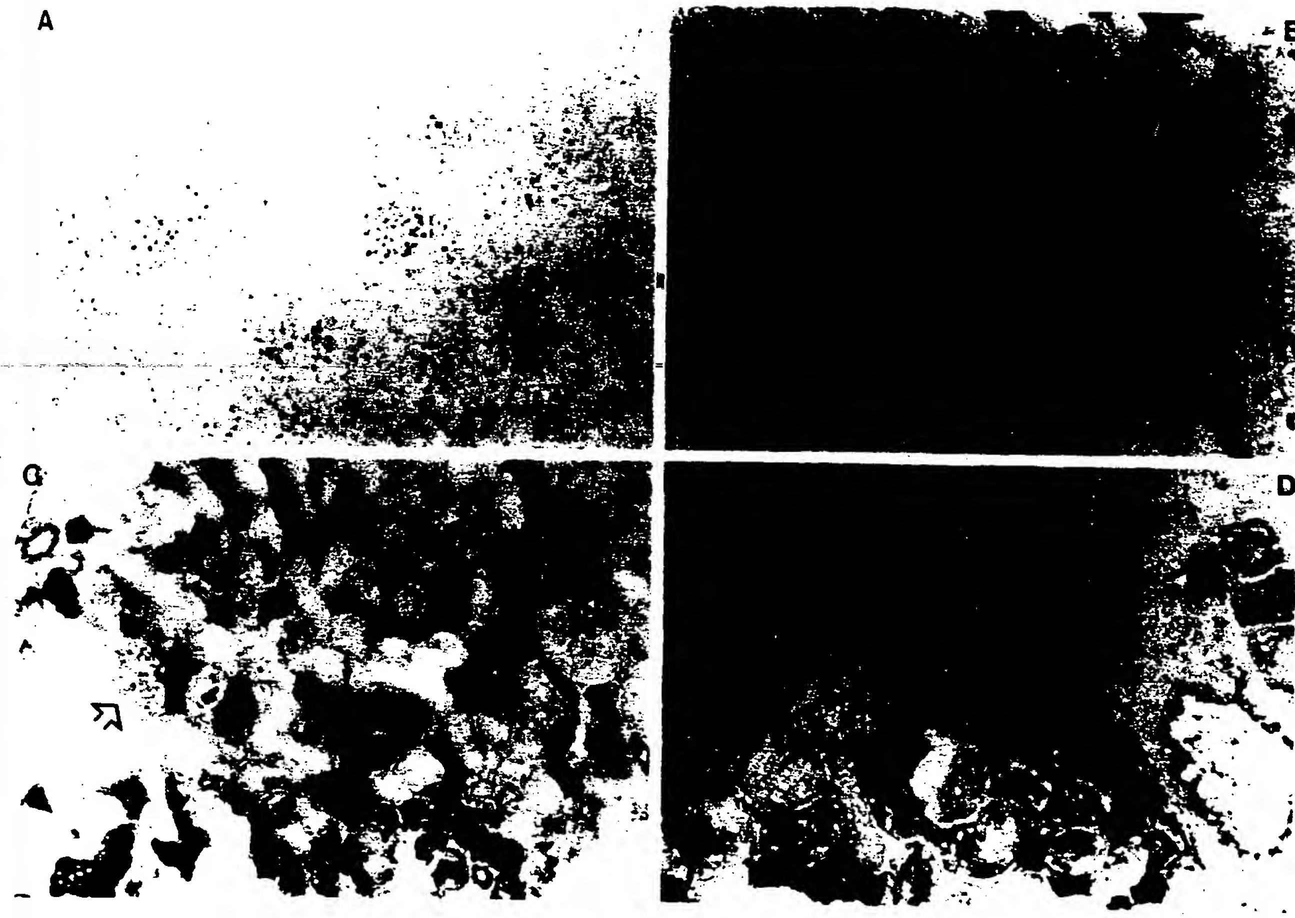

Figure 1. Localization of Ly-6 in normal mouse renal cortex. (A) A.D2-Ly-6b renal cortex stained with PBS as a negative control. (×100). (B) CAF1 renal cortex showing staining of distal tubules (long closed arrow) with YE3 (×100). (C and D) A.D2-Ly-6b renal cortex showing staining of distal tubules (long closed arrow) and of arteriolar endothelium (short closed arrow) with YE3 (×100 and ×160, respectively). Note that glomeruli do not stain (open arrow).

cortical rather than medullary (Figures 2 and 3). It also predominantly involved distal tubules and collecting ducts. Staining with the 34-11-3 mAb, which only detects Ly-6A.2, was negative as expected in these mice.

Ly-6C.2 could not be detected in the kidneys of A.D2-Ly-6b mice with the 34-2-11 mAb. Staining with peroxidase-labeled goat anti-rat antibody and normal mouse serum was completely negative (Figure 1). Staining with peroxidase-labeled goat anti-mouse antibody was mildly positive only in the glomeruli, as has previously been described (39). Class I staining was negative (same as control) with the H-2K, H-2D, and M1 mAb (Figure 1).

We then examined the expression of Ly-6 RNA in the normal kidney. High steady-state RNA levels for Ly-6 were detected in the kidney, compared with the liver, heart, spleen, brain, and other organs in normal mice from both strains studied (Figure 4). Specific oligonucleotide probing showed that both Ly-6A and Ly-6C RNA were present in the kidney (data not shown).

In view of the intense tubular epithelial Ly-6 expression noted on mAb staining, the possibility that this represented tubular resorption as distinct from synthesis of Ly-6 was considered. If epithelial or endothelial cells are the main site of renal Ly-6 synthesis, then renal Ly-6 RNA levels should be radioresistant, whereas if bone marrow–derived cells were the main source, renal Ly-6 RNA levels should be radiosensitive. Ly-6 steady-state RNA levels in normal kidney were unaffected by doses of radiation from 500 to 2,000 rads. Although this result is not conclusive, it suggests that renal Ly-6 production is mainly by epithelial, endothelial, and other nonmarrow–derived cells (Figure 5).

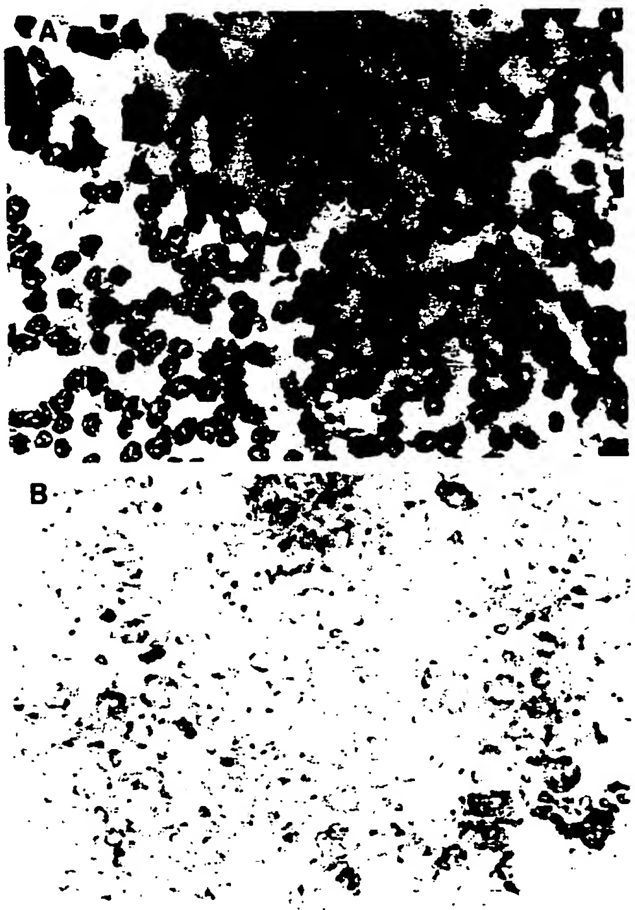

Figure 2. Localization of Ly-6 in mouse renal medulla showing allele-specific variation in expression. (A) Renal medulla from A.D2-Ly-6b mouse (Ly-6.2 haplotype) showing staining of collecting ducts (closed arrow) with YE3 (×100). (B) Renal medulla from CAF1 mouse (Ly-6.1 haplotype) showing no staining with YE3 (×160).

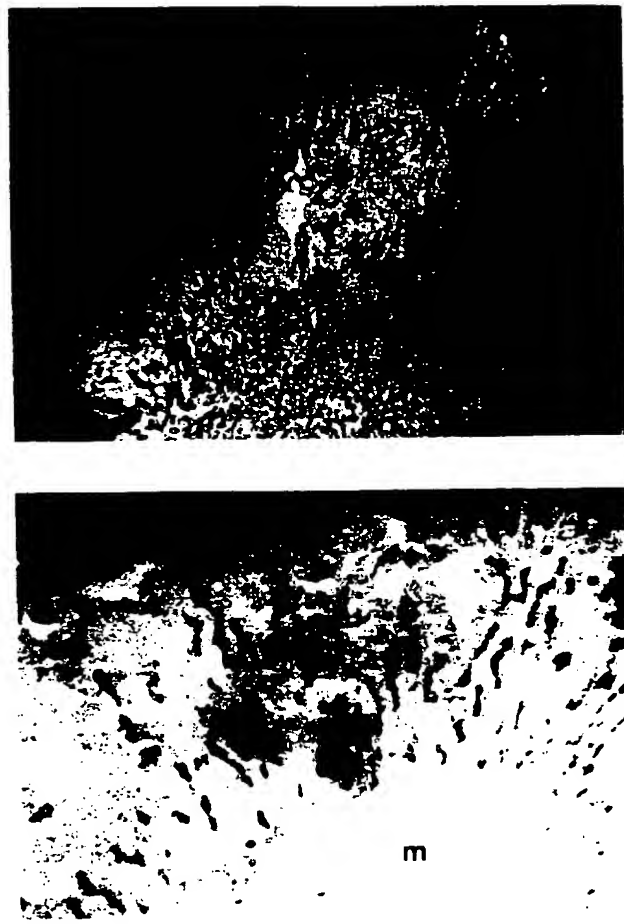

Figure 3. Whole-kidney cross-sections (×25) showing allele-specific variation in the distribution of expression of Ly-6 between cortex (c) and medulla (m) in the A.D2-Ly-6b mouse (Ly-6.2 haplotype) (A) and in the BALB/c mouse (Ly-6.1 haplotype) (B).

IFN-γ Induction of Ly-6

Because the induction of Ly-6 expression by IFN-γ has been reported in lymphocytes (21), we next explored the possibility of such regulation in renal epithelium and endothelium *in vivo*. Recombinant IFN-γ and the various IFN-inducing agents had similar effects on the distribution of Ly-6A.2/E.1 in kidney tissue. On IIP staining, increased intensity of endothelial and tubular expression was seen in all murine strains tested. There was persistence of the pattern of mainly medullary tubular expression in the Ly-6A.2 strain and of mainly cortical tubular expression in the Ly-6E.1 strains (Figure 6). The most notable effect of IFN-γ was in the renal cortex, where proximal tubular epithelial staining became markedly more prominent and more widespread in all strains and took on a predominantly brush border pattern of distribution within the epithelium (Figure 6). No cellular infiltrate was seen, and glomerular and interstitial staining remained negative. Staining for Ly-6A.2 was again appropriately negative in the Ly-6.1 murine strains. Ly-6C.2 could not be detected in IFN-induced kidneys from A.D2-Ly-6b mice with the 34-2-11 mAb.

Staining with anti-class I mAb was strongly positive in the expected distribution on the vascular endothelium and on the antiluminal surface of the proximal tubular epithelium (Figure 6) (45), thus confirming the efficacy of the recombinant IFN-γ and of the various IFN-inducing agents.

Injections with recombinant IFN-γ markedly increased the expression of steady-state RNA levels for Ly-6. The time course of the increase showed that the response is early, being maximal at 6 h and back to baseline levels by 48 h (Figure 7). As measured by densitometry, the increase in expression is 11-fold at 6 h and 7-fold at 24 h. Specific oligonucleotide prob-

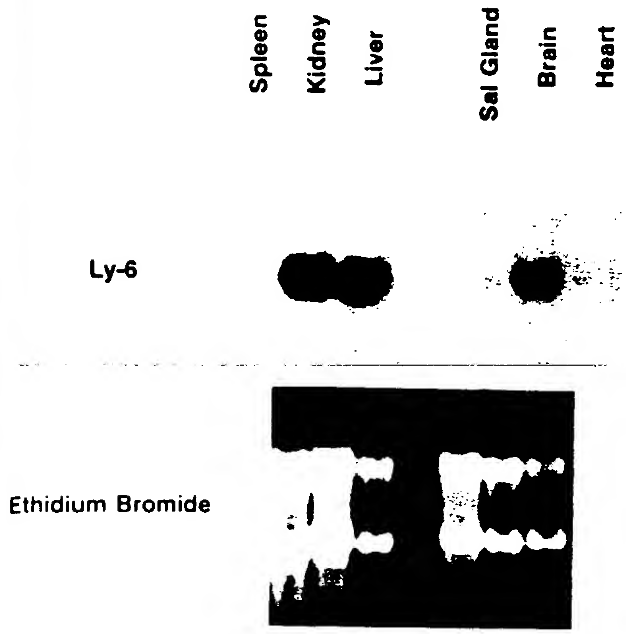

Figure 4. Ly-6 in kidney and other parenchymal organs. Northern blot analysis was done with 15 μg of kidney RNA and probed for Ly-6 (three mice per group). RNA loading is controlled for by staining the gel with ethidium bromide.

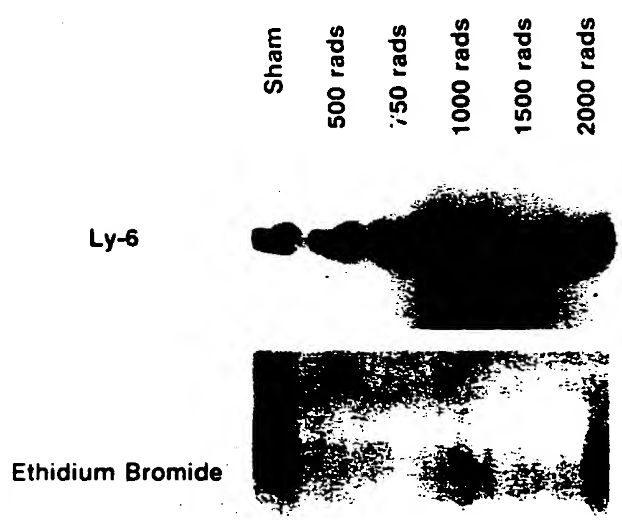

Figure 5. Ly-6 in the kidney is unaffected by radiation. Northern blot analysis was done with 30 μg of kidney RNA from mice treated with radiation doses of 500, 750, 1,000, 1,500 and 2,000 rads (three mice per group). RNA loading is controlled for by staining the gel with ethidium bromide.

ing showed that Ly-6A and Ly-6C expression is increased by IFN-γ.

Changes in Ly-6 Expression in Renal Inflammation

We then asked whether the same effect of IFN-γ on Ly-6 expression occurred in two *in vivo* models of murine renal disease in which IFN-γ is known to play a role. We examined Ly-6 in the MRL lpr/lpr model of lupus nephritis and in mercuric chloride nephropathy. There was no difference in renal Ly-6 expression by immunoperoxidase staining between MRL lpr/lpr mice and +/+ controls at 6 wk of age, with both showing the same distribution of Ly-6 described for normal mice expressing the Ly-6.2 haplotype (Figure 8). Similarly, there was no difference in steady-state RNA levels at 6 wk by Northern blotting (Figure 9). However, at 17 wk, when lymphocytic infiltration is apparent in the kidneys of lpr/lpr mice (35), immunoperoxidase staining showed a marked increase in Ly-6 expression in these mice compared with that in the +/+ controls (Figure 8). This increase was most apparent in the proximal tubules, where it adopted the same luminal pattern described in the IFN-γ–treated mice. It was also apparent that the lymphocytic infiltrate in the kidneys of these mice expressed Ly-6. Similarly, at 17 wk, lpr/lpr kidneys showed markedly increased Ly-6 steady-state RNA levels compared with +/+ kidneys (Figure 9). The difference was 15-fold by densitometry (Figure 9). In view of the Ly-6–positive lymphoid infiltrate, we cannot attribute the increase in renal Ly-6 RNA levels solely to increased epithelial and endothelial Ly-6 production.

In mercuric chloride nephropathy, increased Ly-6 expression by mAb staining, compared with that in sham-treated mice, was seen in the kidneys of mice who received 2 and 3.2 mg/kg, but not in the kidneys of those that received 1.6 mg/kg, three times weekly for 1 wk. Again, the increased expression was most apparent in the proximal tubules in a luminal distribution (Figure 7). In this model, no lymphoid infiltrate was evident at 1 wk. Steady-state RNA levels for Ly-6 were increased, relative to those in kidneys from sham-treated animals, in kidneys from mice treated with 2.0 mg/kg but not in those from mice treated with 1.6 mg/kg of mercuric chloride. The increase was less pronounced than that seen in the lupus nephritis model, being 2.5-fold by densitometry (Figure 9). The absence of significant lymphocytic infiltration in these mice suggests that there is increased renal epithelial and endothelial Ly-6 production in this model. Kidneys from mice treated with 3.2 mg of mercuric chloride per kg actually showed decreased Ly-6 RNA levels relative to those from sham-treated mice, presumably because of the ATN seen with this dose of mercuric chloride (43) (Figure 9).

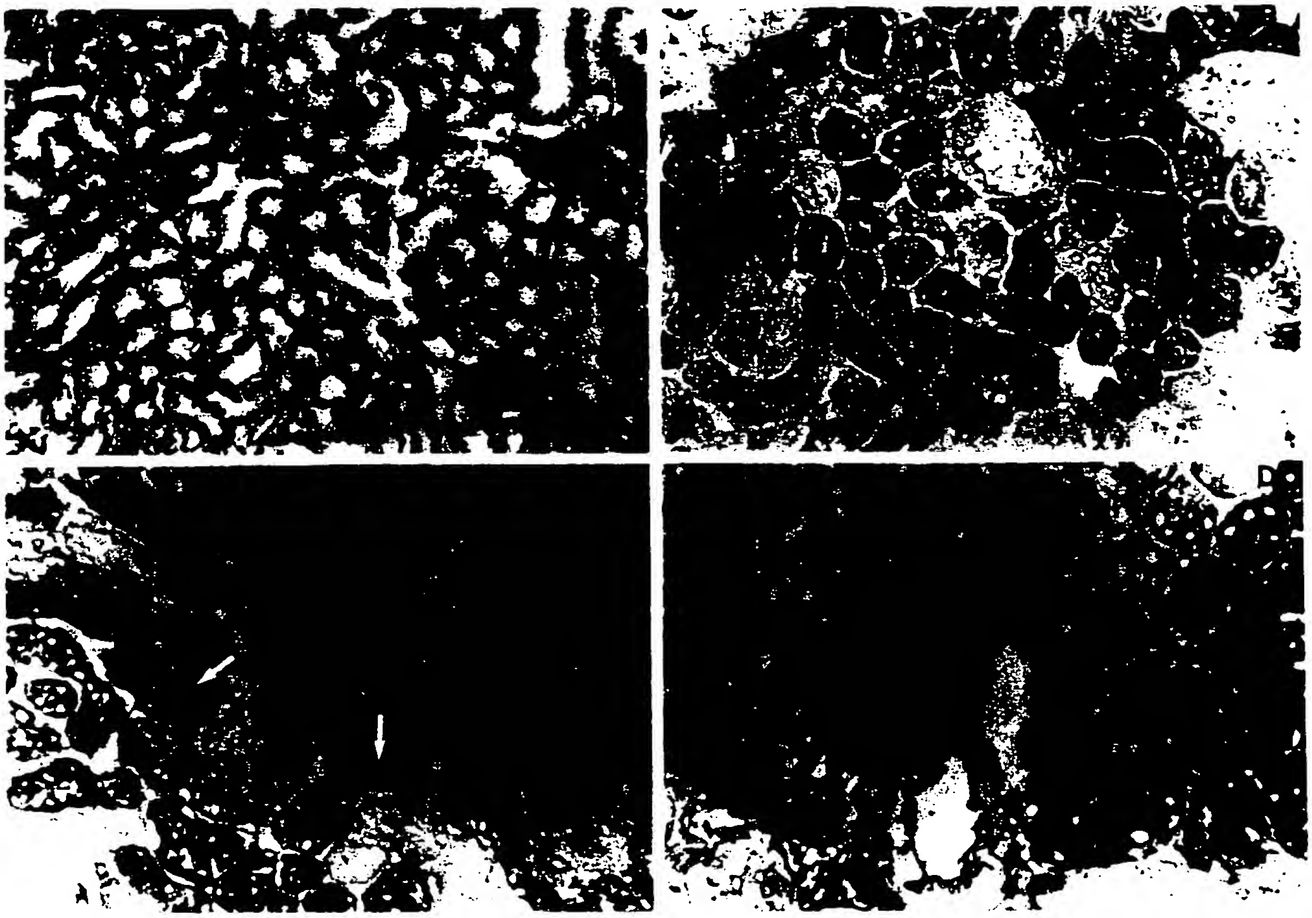

Figure 6. Localization of Ly-6 in the mouse kidney after IFN induction with lipopolysaccharide. (A) Renal cortex from A.D2-Ly-6b renal cortex stained with M1 as a positive control. The staining is mainly on the proximal tubules in a basolateral distribution (arrow) (×100). (B) A.D2-Ly-6b renal cortex stained with YE3. The staining is on both the proximal (closed arrow) and distal tubules (closed arrowhead) and in the former takes on a luminal distribution (×100). (C and D) Staining of A.D2-Ly-6b renal cortex with YE3 under higher power (×160 and ×400, respectively). Proximal tubular staining shows a luminal pattern of distribution (white arrows). Endothelial staining is also prominent (open arrow).

DISCUSSION

We have shown, using both mAb and cDNA probes, that Ly-6 A.2/E.1 is strongly expressed in the kidney on both the tubular epithelium and the vascular endothelium, is radiation resistant, and is up-regulated by IFN-γ. The endothelial expression is on the small arteries, arterioles, and veins but not on the glomeruli or capillaries. It does not differ from the endothelial expression of Ly-6 in other organs. In contrast, the intense expression in the renal epithelium is unique. It is allele specific in that its pattern of distribution between the cortex and the medulla varies markedly between murine strains expressing the two distinct Ly-6 alleles, A.2 and E.1. This seems surprising, but a similar variation in expression on peripheral lymphocytes has been reported for the same two alleles (1,2). Renal expression is also locus specific in that it appears to be accounted for predominantly by Ly-6A.2 and Ly-6E.1 rather than by Ly-6C. Again, similar variations in expression between A/E and C have been reported in lymphoid tissue (1,2). The mechanisms underlying these differences in gene expression are unknown.

The induction of Ly-6 in the kidney by IFN-γ occurs rapidly and is not related to any interstitial infiltrate. Rather, it appears to be due to increased intensity of both epithelial and endothelial expression. The presence of the same pattern of up-regulation in two models of murine renal disease in which IFN-γ is known to play a role indicates that the IFN-γ regulation of Ly-6 has *in vivo* relevance. The IFN regulation of Ly-6 has been described in lymphocytes (20,21), and an IFN-responsive sequence, analogous to that found in association with MHC class I and II genes, has been identified upstream of one of the sucessfully sequenced Ly-6 genes (18). Other IFN-inducible structures in the kidney include MHC class I and II,

Figure 7. IFN-γ induction of Ly-6 in the kidney. Northern blot analysis was done with 30 μg of kidney RNA from mice 6, 24, and 48 h after IFN-γ administration and probed for Ly-6 (three mice per group). RNA loading is controlled for by probing the same blot for glyceraldehyde phosphate dehydrogenase.

β-2-microglobulin, and the intracellular adhesion molecule ICAM-1 (45,46).

The predominantly distal expression of Ly-6 in the nephron contrasts with that of other immunologically important antigen systems expressed on the tubules, such as MHC class I and II and ICAM-1, which have a predominantly proximal distribution (45,46). In addition, when proximal tubular Ly-6 expression does become prominent after IFN-γ induction, it follows a luminal or brush border pattern in contrast to the mainly cytoplasmic pattern of distribution seen in the noninduced kidney. The cytoplasmic expression is presumed to represent Ly-6 proteins before or during the attachment of the GPI anchor and the subsequent transport to the apical surface of the epithelium. The luminal expression of IFN-induced Ly-6 is similar to the pattern found with ICAM-1 (47) but contrasts with the IFN-induced pattern for classical MHC class I and II antigens, which is antiluminal, *i.e.*, basolateral (45). There is at least one class I–encoded molecule, Qa-2, that has a GPI

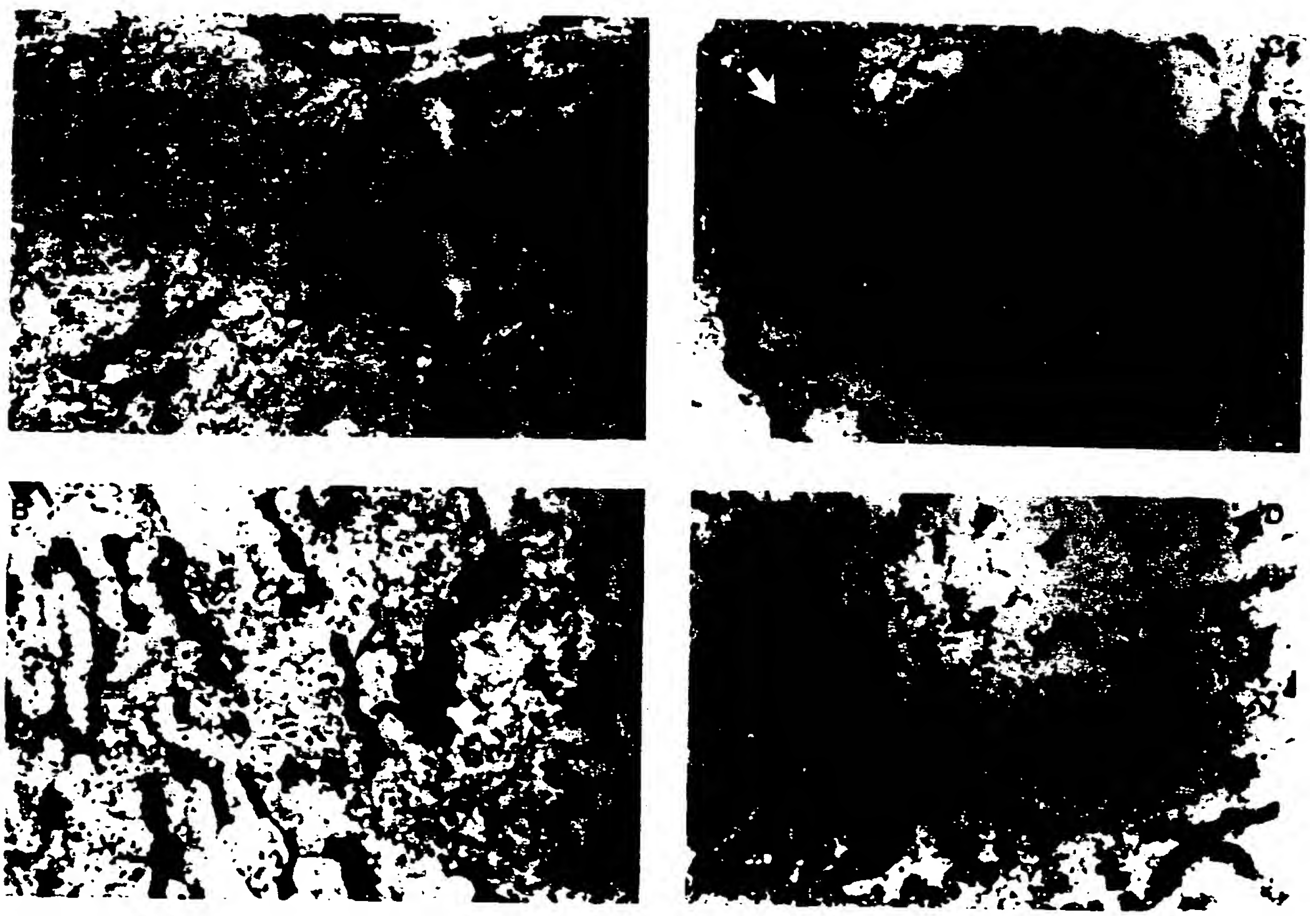

Figure 8. Ly-6 expression in murine renal disease. (A) Kidney section from MRL lpr/lpr mouse at 6 wk. (B) Kidney section from MRL +/+ mouse at 6 wk. Both show tubular epithelial staining with YE3 (closed arrows). (C) Kidney from MRL lpr/lpr mouse at 17 wk, showing staining of lymphocytic infiltrate (white arrow) and of tubular epithelium in a luminal pattern of distribution (closed arrow). (D) Control kidney from MRL +/+ mouse (×100).

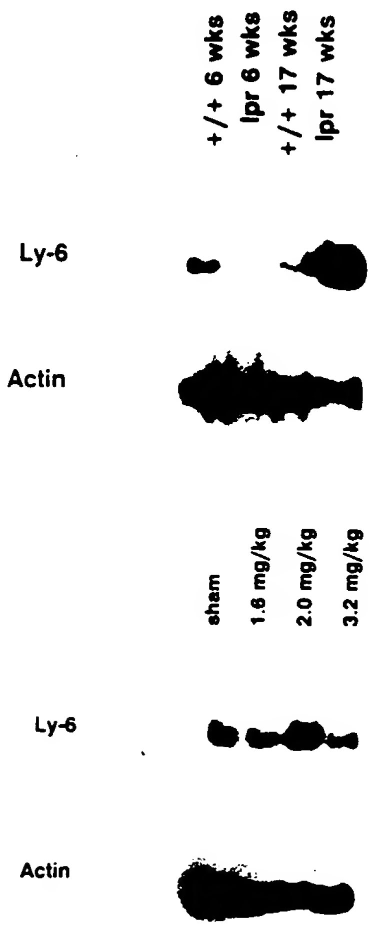

Figure 9. Increase in Ly-6 in murine renal disease. Above is Northern blot analysis done with 15 μg of kidney RNA from MRL +/+ and lpr/lpr mice at 6 and 17 wk (three mice per group), and below is Northern blot analysis done with 15 μg of RNA from mice receiving sham treatment or mercuric chloride at 1.6, 2.0, and 3.2 g/kg three times weekly for 1 wk (three mice per group). In both cases, β-actin probing of the same blots is used to control for RNA loading.

anchor (48), but it is unknown whether it has any renal epithelial expression. Interestingly, it has recently been demonstrated in a renal epithelial cell line that membrane proteins with a GPI anchor are always expressed on the apical, and never on the basolateral, surface of the cell, suggesting that attachment of the GPI anchor to the precursor protein in the endoplasmic reticulum leads to specific apical targeting of the resulting product (49,50). Ly-6 antigens all have a GPI anchor, so it is not surprising that Ly-6A.2 and Ly-6E.1 localize to the luminal surface after IFN-γ induction. Some adhesion molecules, such as LFA-3, have both transmembrane peptide domains and the capacity to be GPI anchored; however, the amino acid sequences of known Ly-6 proteins do not include any transmembrane domains, and therefore, this does not appear to be the case for Ly-6. Specific enzymatic digestion experiments to prove that renal Ly-6 is attached to the apical tubular epithelium by a GPI anchor have not been done.

Multigene systems analogous to Ly-6 have been described in the sheep and rat (51,52), and two human GPI-anchored cell surface proteins with homology to Ly-6 have recently been identified (29,31). These are CD59 and uPAR. CD59 is of particular interest because, like Ly-6, it is extensively expressed on the distal tubular epithelium and on the vascular endothelium of the kidney (32,33). The function of CD59 is thought to be homologous restriction of the cytolytic action of the membrane attack complex of complement (53). CD59 mAb are, like Ly-6 mAb, capable of mediating T cell activation (54). In view of these similarities between Ly-6 and CD59 in structure, signal transduction capabilities, and renal expression, it seems reasonable to speculate that CD59 is the human equivalent of Ly-6. However, CD59 differs from Ly-6 in being encoded by a single gene rather than by a multigene family, in being polymorphic, and in not being IFN regulated (55). Amino acid homology between these two proteins is only 25%, and their tissue distribution differs in that Ly-6, unlike CD59, is not detected on red blood cells. Also, the capacity to mediate T cell activation appears to be a characteristic of all GPI-anchored membrane proteins and not just of Ly-6 and CD59 (56). It may therefore be safer to conclude that Ly-6 and CD59 are relatives rather than exact equivalents (55).

Clues to the identify of the physiologic ligand for Ly-6 may be derived from the structures of known ligands for related molecules. The natural ligands for CD59 and uPAR are the C5–C8 complex of the complement system and urokinase, respectively. Each of these has a receptor-binding region with homology to the growth factor module, initially described in the epidermal growth factor molecule but also found in a variety of other molecules, including coagulation factors IX and X, transforming growth factor-alpha, low-density lipoprotein receptor, and selectin adhesion molecules (31,57). Palfree has recently speculated that the natural ligand for Ly-6 may similarly contain an epidermal growth factor module, and he has noted that one such molecule is Tamm-Horsfall glycoprotein (THGP), which is present in normal urine (31,58). This is of interest in view of our findings (*1*) that Ly-6 expression is prominent on the renal tubules, especially distally where THGP is most abundant, and (*2*) that IFN-γ–induced Ly-6 is mainly on the apical surface of the tubular epithelium, where it is exposed to potential luminal ligands, such as THGP, rather than to blood-borne ones. THGP has no clearly proven physiologic function, although it is known to have immunosuppressive properties (58).

A potential interaction between it and renal tubular Ly-6 merits investigation.

Finally, the strong antigenicity of Ly-6, its regulation by IFN-γ, its polymorphism, and its presence on the endothelium, as well as the allele-specific variation in its expression, make it a unique candidate to be an important antigen system in transplantation. There has long been speculation about a non-MHC endothelial antigen system that might evoke destructive antibody responses in allograft rejection, but no such system has been convincingly characterized (59). Ly-6 merits consideration in this context.

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